Histamine H_3 receptor-mediated depression of synaptic transmission in the dentate gyrus of the rat *in vitro*

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1. The effects of histamine on excitatory synaptic transmission in the dentate gyrus region of rat hippocampal slices were examined using extracellular and whole-cell patch-clamp recording techniques. The $GABA_A$ receptor antagonist picrotoxin (50 μM) was present in the bath in all experiments.

2. Histamine (0·7–70 μM) reversibly depressed field excitatory postsynaptic potentials (fEPSPs) or excitatory postsynaptic currents (EPSCs) recorded intracellularly by up to 30%. The presynaptic fibre volley and EPSC reversal potential were unaffected by histamine, as were responses following pressure ejection of the glutamate receptor agonist *S*-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (*S*-AMPA) into the slice.

3. Histamine (7 μM) depressed equally the AMPA and *N*-methyl-D-aspartate (NMDA) components of the dual-component EPSC, recorded at −40 mV.

4. In addition to depressing synaptic transmission, histamine also reduced the magnitude of paired-pulse depression (PPD; 40 ms interpulse interval) of the medial perforant path EPSC.

5. Histamine depressed medial perforant path EPSCs more strongly than lateral perforant path EPSCs. Paired-pulse facilitation (PPF; 40 ms interpulse interval) in the lateral perforant path was enhanced by histamine.

6. The effects of histamine on synaptic transmission and PPD were mimicked by the selective H_3 receptor agonist *R*-α-methylhistamine (0·1–10 μM) but not by the selective H_2 receptor agonist dimaprit (10 μM). Similarly, the H_3 receptor antagonist thioperamide (10 μM) blocked the effect of histamine whereas the H_1 antagonist mepyramine (1 μM) and the H_2 receptor antagonist cimetidine (50 μM) were ineffective.

7. Histamine actions on synaptic transmission and PPD were not occluded by application of the metabotropic glutamate agonist L-2-amino-4-phosphonobutyrate (AP4).

8. The results indicate that histamine depresses synaptic transmission in the dentate gyrus by binding to histamine H_3 receptors located on perforant path terminals. The mechanism by which histamine depresses transmission is independent of that used by class III metabotropic glutamate receptors.

In the vertebrate central nervous system the neuromodulator histamine is released from neurons whose cell bodies are located in the tuberomammillary nucleus of the hypothalamus (Wada, Inagaki, Yamatodani & Watanabe, 1991). These neurons send out axons which project to the whole brain in a diffuse manner, contacting neurons, as well as astrocytes and blood vessels. In accordance with the anatomy of the histaminergic neurons, histamine has been proposed to be involved in arousal mechanisms, in the control of the sympathetic nervous system, in thermoregulation and in drinking behaviours (Hough 1988; Lin, Sakai & Jouvet, 1988; Wada *et al.* 1991).

Histamine, released from axonal varicosities, acts upon three types of receptors. Histamine H_1 receptors are coupled to phospholipase C and thus to the release of the second messengers inositol trisphosphate and diacylglycerol, whilst H_2 receptors are coupled positively to adenylate cyclase (Schwartz, Arrang, Garbarg, Pollard & Ruat, 1991). Both of these receptors are located postsynaptically. In contrast, the histamine H_3 receptor, which was first described by Arrang, Garbarg & Schwartz (1983), is a presynaptic autoreceptor which controls histamine release. In addition to being an autoreceptor, the H_3 receptor is also a heteroreceptor, i.e. it controls the release of other neurotransmitters. To date H_3 receptors have been shown to influence the release of serotonin, noradrenaline and acetylcholine in the brain (Schlicker, Betz & Göthert, 1988; Schlicker, Fink, Hinterthaner & Göthert, 1989; Arrang,

Drutel & Schwartz, 1995). They have not, however, so far been shown to modulate glutamate release.

In the hippocampal formation, histamine has a number of prominent effects on the principal cells. Activation of the histamine H_1 receptor leads to hyperpolarizations whereas H_2 receptor stimulation produces an increase of the intracellular cAMP concentration, depolarization and a blockade of the calcium-dependent potassium conductance in both pyramidal and granule cells (Greene & Haas, 1990; Haas, 1992). Effects attributable to histamine H_3 receptor activation have not so far been described. Recently, we have shown that histamine can enhance *N*-methyl-D-aspartate (NMDA) receptor-mediated synaptic potentials and can facilitate the induction of long-term potentiation in the CA1 region (Brown, Fedorov, Haas & Reymann, 1995; Yanovsky, Reymann & Haas, 1995).

In this study we show that histamine, acting through H_3 receptors can depress synaptic transmission in the dentate gyrus by a presynaptic mechanism and can, therefore, also modulate glutamate release. In the hippocampal formation the dentate gyrus receives the strongest histaminergic innervation (Panula, Pirvola, Auvinen & Airaksinen, 1989) and also contains the highest density of H_3 receptor binding sites (Pollard, Moreau, Arrang & Schwartz, 1993).

METHODS

Hippocampal slices were prepared from 7- to 8-week-old, male Wistar rats (Institute of Neurobiology, Magdeburg, Germany; breeding stock) as described previously (Brown & Reymann, 1995). The animals were killed rapidly by a blow to the back of the neck using a metal rod and then immediately decapitated. Transverse slices, 400 μm thick, were cut using a tissue chopper, transferred to a recording chamber and left to recover for 1 h at 32 °C in normal artificial cerebrospinal fluid (ACSF), which contained (mM): NaCl, 124; KCl, 2·8; KH_2PO_4, 1·2; $MgSO_4$, 4; $CaCl_2$, 4; $NaHCO_3$, 25·6; D-glucose, 10; and picrotoxin, 0·05. Constant voltage bipolar pulses (1–10 V, 0·2 ms duration) were applied every 30 s through a monopolar, lacquer-coated, platinum electrode placed in either the middle or outer third of the stratum moleculare of the dentate gyrus to stimulate the medial or lateral perforant path inputs, respectively. Paired pulses were delivered with an interstimulus interval (ISI) of 40 ms. For recording of field excitatory postsynaptic potentials (fEPSPs) a glass electrode filled with ACSF was inserted into the molecular layer at the same level as the stimulation electrode. The initial slope function of the fEPSP (fEPSP SF) was used as the measure of this potential. The medial or lateral perforant path was distinguished according to the kinetics of the synaptic potentials and the presence of paired-pulse depression or facilitation, respectively (McNaughton, 1982; Keller, Konnerth & Yaari, 1991; Hanse & Gustafsson, 1992; Andreason & Hablitz, 1994).

For pressure ejection of *S*-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (*S*-AMPA), an extracellular recording electrode was filled with *S*-AMPA (200 or 500 μM) dissolved in ACSF. The tip of the electrode was carefully broken on the net supporting the slices and then inserted into the stratum moleculare to record both fEPSPs and field responses to AMPA (Fedorov & Reymann, 1993). AMPA was injected into the slice by short-duration pressure pulses (16–26 ms, 2 atm); two or three responses to AMPA with an interval of 40 s between applications were averaged every 5 min. Three test stimuli (responses averaged) with an interstimulus interval of 20 s were applied interleaved with the AMPA applications to monitor synaptic responses. At the end of each experiment, 100 μl of 1·5 mM 6-nitro-7-sulphamoylbenzo(*f*)-quinoxaline-2,3-dione (NBQX; approximately 10 μM final concentration) was applied into the chamber to demonstrate that the responses were due to activation of AMPA receptors. In some cases a small, transient positive or negative deflection remained following NBQX (pressure artifact – Fedorov & Reymann, 1993), which was easily distinguishable from the 'genuine' responses.

Intracellular recordings were made from dentate granule cells using the 'blind' whole-cell patch-clamp technique (Staley, Otis & Mody, 1992). Intracellular signals were recorded using an SEC-10L single-electrode current and voltage-clamp amplifier (npi, Tamm, Germany) using a switching frequency of 40–48 kHz at a duty cyle of 25%. Patch pipettes (3–7 MΩ) were normally filled with an intracellular solution containing (mM): potassium gluconate, 135; KCl, 5; $MgCl_2$, 2; Hepes, 10; and D-glucose, 20 (pH 7·2 with KOH). Excitatory postsynaptic currents (EPSCs) were recorded at a holding potential of −75 mV unless otherwise stated. For experiments in which it was necessary to hold the neuron away from its resting membrane potential (measurement of reversal potential or dual-component EPSCs), patch pipettes were filled with a solution containing (mM): caesium methanesulphonate, 135; CsCl, 5; $MgCl_2$, 2; NaCl, 10; $CaCl_2$, 1; EGTA, 10; Hepes, 10; and lidocaine *N*-ethyl bromide quaternary salt (QX-314), 10. With this solution, it was necessary to wait for 15 min after obtaining access to the whole cell for the input resistance and EPSC amplitude to stabilize – this was probably due to slow wash-in of QX-314. Access resistance was calculated from the instantaneous current measured at the beginning of a 5 mV, 100 ms hyperpolarizing step from the holding potential and input resistance from the steady-state current remaining at the end of the step. For potassium gluconate-filled pipettes access resistances were 39 ± 2 MΩ and input resistances 148 ± 10 MΩ ($n = 22$) whereas for caesium methanesulphonate-filled electrodes access resistances were 37 ± 3 MΩ and input resistances (measured from a holding potential of −40 mV) were 304 ± 41 MΩ ($n = 15$). Neurons with resting membrane potentials more negative than −60 mV and input resistances greater than 50 MΩ were accepted for analysis. Experiments in which the access resistance changed by more than 20% during the course of the experiment or the EPSCs were not stable prior to drug application were discarded.

Drugs used in this study were: histamine dihydrochloride (Sigma), *R*-α-methylhistamine dihydrochloride (RBI), dimaprit dihydrochloride (RBI), mepyramine (Sigma), cimetidine (Sigma), thioperamide maleate (RBI), D-2-amino-5-phosphonovaleric acid (AP5; Tocris Cookson, Bristol, UK), L-2-amino-4-phosphonobutyrate (AP4; Tocris Cookson), QX-314 (Alomone Lab.), picrotoxin (Sigma), *S*-AMPA (Tocris Cookson) and NBQX (Tocris Cookson). All other chemicals were obtained from Sigma. Drugs were dissolved in distilled water, titrated to pH 7·4 using NaOH and stored as stock solutions. Histamine, dimaprit and *R*-α-methylhistamine dihydrochloride were applied as a one-time bolus injection (indicated in the figures by a vertical arrow) into a column immediately preceding the chamber, where the ACSF was gassed with carbogen; 100 μl of the drug was applied. The effective concentration of the drug was calculated as $C \times (0{\cdot}1/15)$ where C is the concentration of the drug applied, based on a total volume of

the bubbling column and chamber of 15 ml. All values given in the text and figure legends refer to this calculated concentration. Other drugs were bath applied. Percentage paired-pulse depression (PPD) or paired-pulse facilitation (PPF) was calculated as: Percentage PPD = (EPSP slope, 2nd pulse/EPSP slope, 1st pulse) × 100. The coefficient of variation (CV) of synaptic responses was calculated as the standard deviation/mean. All values are given as means ± S.E.M. Statistical comparisons were made using Student's *t* test.

RESULTS

The results presented in this paper are based on fEPSPs recorded from sixty-six slices and whole-cell EPSCs (100–400 pA in amplitude) recorded from thirty-seven granule cells. The $GABA_A$ receptor antagonist picrotoxin (50 μM) was present in the bath in all experiments to isolate excitatory postsynaptic potentials/currents. Granule cells had resting membrane potentials of −68 ± 1 mV ($n = 37$).

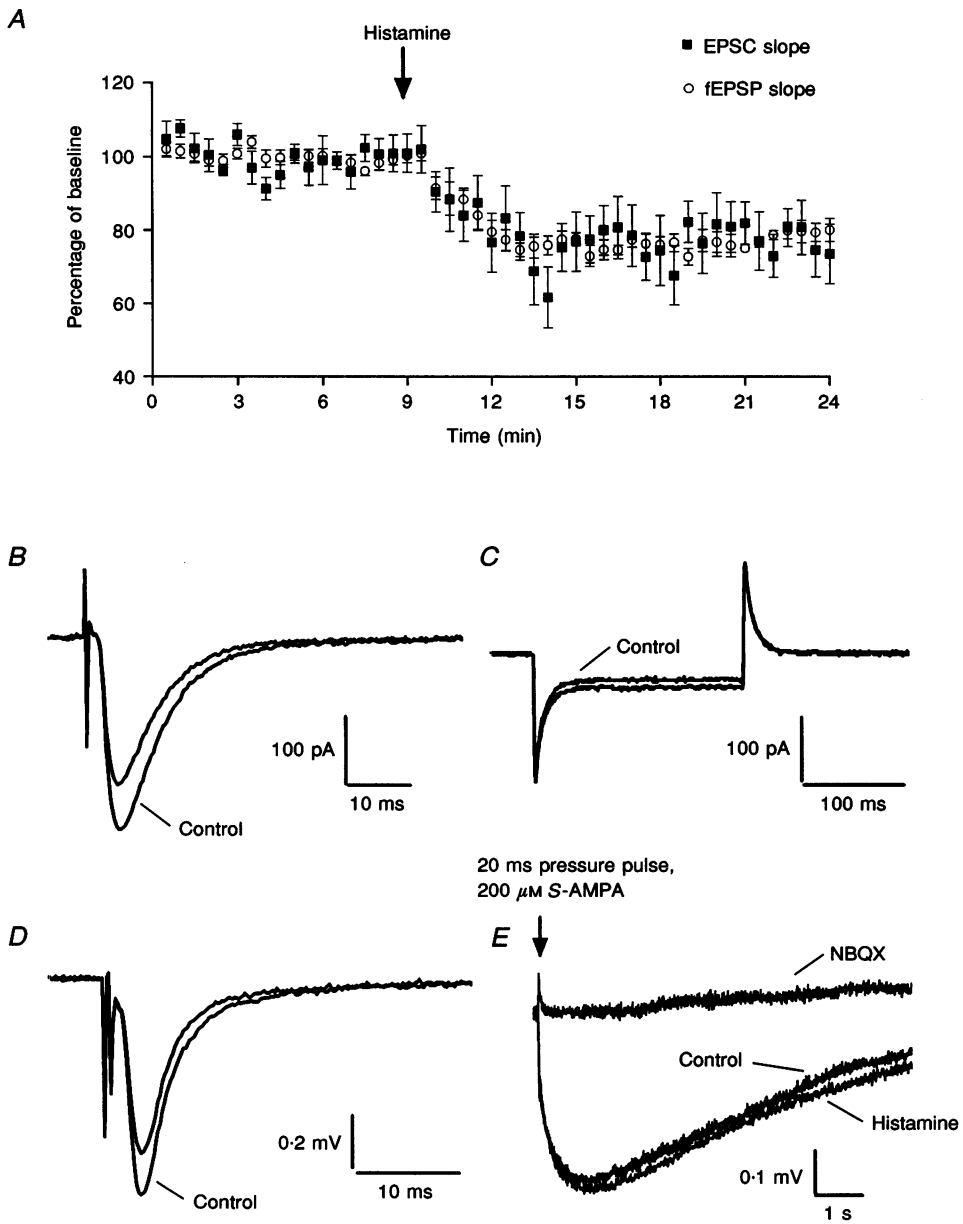

Figure 1. Histamine depresses synaptic transmission in the medial perforant path

A, plot of slope of the EPSC (■, $n = 6$) at a holding potential of −75 mV and the fEPSP (○, $n = 8$) against time. Histamine (7 μM) was applied at the time indicated by the arrow. *B* and *D*, representative analog traces of the EPSC and fEPSP, respectively, in control and following the application of histamine. *C*, current responses following 5 mV hyperpolarizing steps applied before and after histamine application. *E*, field responses recorded following pressure ejection of *S*-AMPA into stratum moleculare. Note that histamine did not depress these responses. The AMPA receptor antagonist NBQX abolished the response, however. The traces in *B*, *C*, *D* and *E* are the averages of 3, 3, 6 and 6 consecutive responses, respectively.

At a holding potential of −75 mV, medial perforant path-evoked EPSCs had rise times (10–90%) of 1·63 ± 0·12 ms and decay time constants (single-exponential decay) of 7·69 ± 0·60 ms ($n = 14$). Lateral perforant path-evoked EPSCs had rise times of 2·46 ± 0·18 ms and decay time constants of 8·43 ± 0·85 ms ($n = 8$). These values are in broad agreement with other studies in this region (Keller *et al.* 1991; Andreason & Hablitz, 1994; O'Connor, Rowan & Anwyl, 1995).

Effects of histamine on synaptic transmission and short-term plasticity

Application of histamine led to a rapid reduction of the initial slope of extracellularly recorded fEPSPs and intracellularly recorded EPSCs following stimulation of the medial perforant path (Fig. 1). This effect was dose dependent. Initial experiments with a high concentration of histamine (70 μM) revealed a depression of the fEPSP slope to 75·0 ± 2·5% of control ($n = 6$) following histamine addition. At 7 μM the depression was to 75·7 ± 3·2% of control ($n = 8$) whilst at 0·7 μM the depression only reached 87·1 ± 2·0% ($n = 5$). Values for the EPSC slope were very similar (Fig. 1). Since 7 and 70 μM histamine produced a similar magnitude of depression, a concentration of 7 μM was used in all subsequent experiments. Although the depression caused by histamine exhibited a rapid onset, it was extremely slow to reverse – 1 h was needed for full recovery following application of 7 μM histamine. The depression of the synaptic response occured in the absence of changes of the presynaptic fibre volley (data not shown). Histamine (7 μM) also did not change the reversal potential of the component of the EPSC mediated by AMPA receptors ($n = 3$, measured in the presence of 50 μM AP5). Furthermore, it was clear from these experiments that the effect of histamine on synaptic transmission was not affected by the duration of the postsynaptic dialysis (up to 30 min) or by the contents of the pipette solution (see Methods), suggesting that histamine exerts its effect presynaptically. Effects on holding current or input resistance were usually very minor and were not correlated in time course or direction with the depression of synaptic currents. Histamine (7 μM) increased the CV of the EPSC slope from 0·082 ± 0·016 in control to 0·140 ± 0·022 ($n = 6$, $P < 0·05$), again consistent with a presynaptic change.

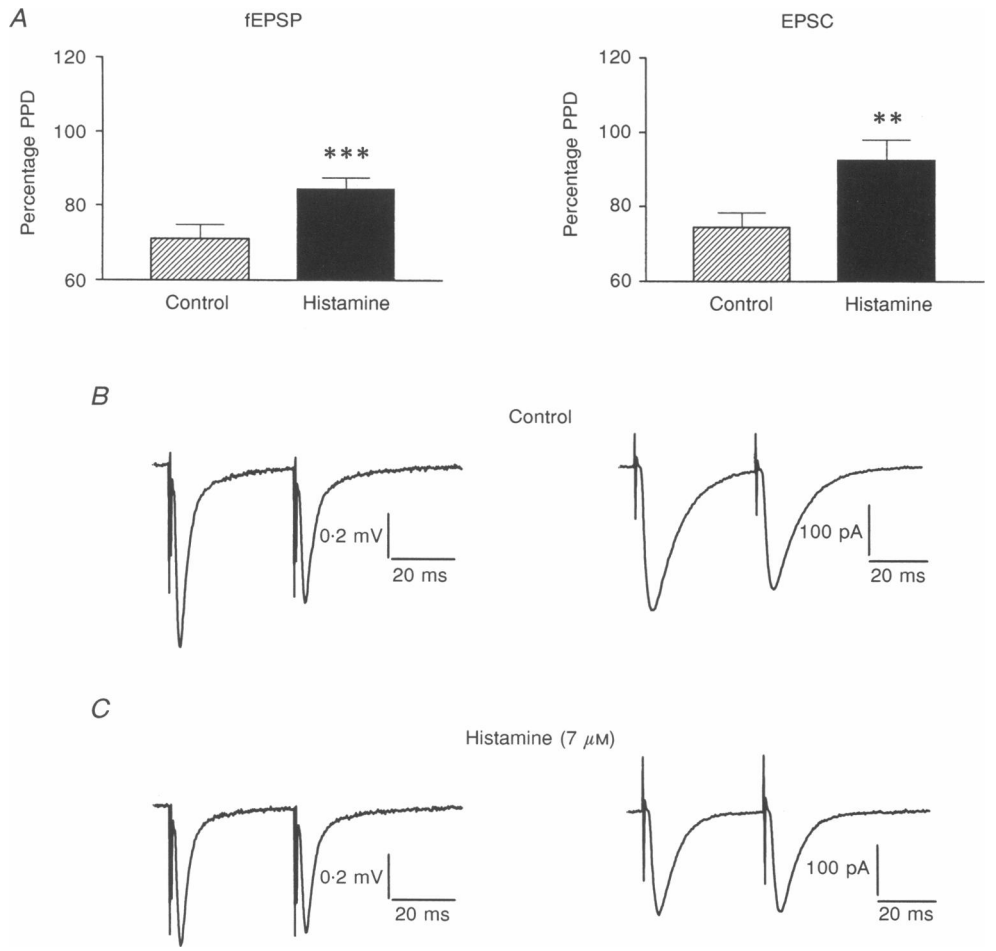

Figure 2. Histamine reduces paired-pulse depression (PPD) in the medial perforant path

Data for the fEPSP are shown on the left side of the figure and data for the EPSC on the right side. *A*, mean data for the fEPSP ($n = 10$) and EPSC ($n = 7$). *** $P < 0·001$, ** $P < 0·01$. *B*, analog traces in control. *C*, analog traces in the presence of histamine.

Three further types of experiments were performed to confirm that histamine was acting presynaptically. The action of histamine on postsynaptic responses to the selective glutamate receptor agonist AMPA, the action of histamine on paired-pulse plasticity and the effect of histamine on dual-component EPSCs were all examined. Responses obtained by ejection of 200 or 500 μM *S*-AMPA into the slice had amplitudes of 0·2–0·4 mV and lasted between 5 and 20 s. Application of 7 μM histamine had no effect on either the amplitude or the area of these responses (Fig. 1*E*), whilst at the same time fEPSPs recorded from the same pipette were clearly depressed. Ten minutes after addition of histamine, the amplitude of the pressure responses was at 105·1 ± 2·4% of baseline and the area of the responses was at 103·5 ± 5·2% of baseline, in contrast to the fEPSP slope, which was depressed to 75·9 ± 3·4% of baseline ($n = 5$). Thus, synaptic responses were depressed whilst direct postsynaptic responses were unaffected.

The medial perforant path input to the dentate gyrus exhibits PPD in response to pairs of stimuli with interstimulus intervals (ISIs) in the range 20–2000 ms (McNaughton, 1982). This form of short-term plasticity appears to have two phases: an early phase, maximal at 40 ms, which can be modified by certain metabotropic glutamate receptor (mGluR) agonists and by carbachol, and a late phase, maximal at 500 ms, which is insensitive to these agents (Kahle & Cotman, 1993; Brown & Reymann, 1995). To see if histamine could also modulate the early phase of PPD we applied pairs of pulses with an ISI of 40 ms, in the absence and presence of histamine. The results of these experiments are presented in Fig. 2. Histamine (7 μM) reduced PPD of the fEPSP slope from 71·1 ± 3·7% in control to 84·4 ± 3·0% ($n = 10$, $P < 0·001$) and PPD of the EPSC slope from 74·5 ± 3·8% in control to 92·5 ± 5·7% ($n = 5$, $P < 0·05$).

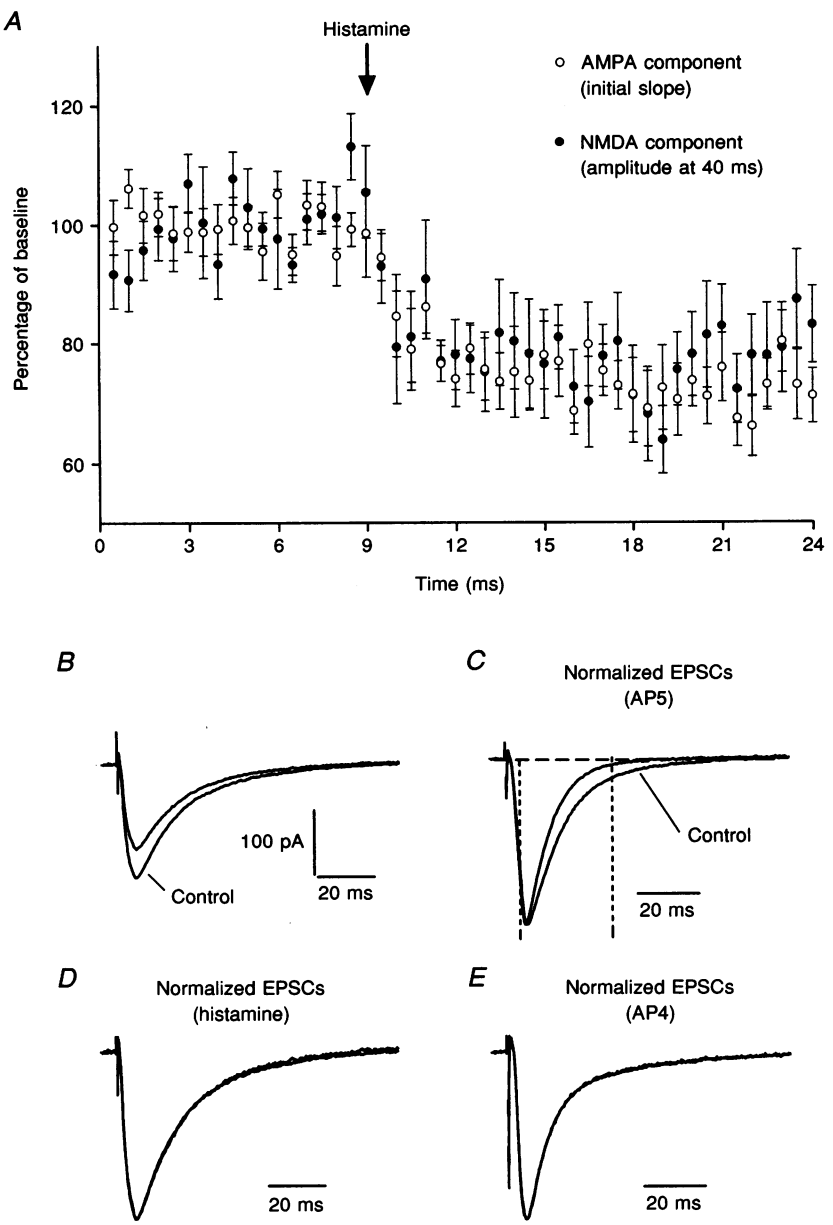

Figure 3. Histamine depresses equally the AMPA and NMDA components of the EPSC

A, time course of the effect of histamine (7 μM; administered at the time indicated by the arrow) on the AMPA (○) and NMDA (●) components of the EPSC ($n = 8$). *B*, analog traces before and after histamine application. *C*, *D* and *E*, analog traces normalized by amplitude in control and in the presence of AP-5 (50 μM), histamine (7 μM) and AP4 (100 μM), respectively. Each trace in *B*–*E* is the average of 18 consecutive responses.

Drugs which reduce transmitter release at glutamatergic synapses are found to reduce the AMPA and NMDA components of the EPSC equally, in the absence of any additional postsynaptic effects. To test if histamine reduces both components of the EPSC equally, dual component EPSCs were recorded at a holding potential of −40 mV. The initial slope of the EPSC was taken as a measure of the AMPA receptor-mediated component and the amplitude of the EPSC at 40 ms was taken as a measure of the NMDA receptor-mediated component. In control experiments, AP5 (50 μM) did not reduce the initial slope of the EPSC but did substantially reduce the amplitude measured at 40 ms ($n = 2$, Fig. 3*C*). In contrast, the mGluR agonist, AP4 (100 μM), which is known to act presynaptically (Baskys & Malenka, 1991), reduced both components in parallel ($n = 2$, Fig. 3*E*). Histamine (7 μM) was found to act in an identical way to AP4, i.e. it reduced both components of the EPSC in parallel (Fig. 3*A*, *B* and *D*). Following histamine application the AMPA component was reduced to 75·2 ± 7·6% of control and the NMDA component to 78·1 ± 5·9% ($n = 8$).

Effects of histamine in the lateral perforant path

Many exogenous and endogenous modulators of synaptic transmission and plasticity in the dentate gyrus have different effects at the lateral perforant path–granule cell synapse than at the medial perforant path–granule cell synapse. Thus, we tested the effects of histamine in this pathway as well. Stimulation of the outer third of the molecular layer elicited a fEPSP which had a mono-exponential decay, in comparison with the bi-exponential decay of the medial perforant path fEPSP. Whole-cell EPSCs had slower rise times and decay times than those evoked by medial perforant path stimulation (see above), consistent with enhanced dendritic filtering of the responses originating in the distal dendrites. Both extracellular fEPSPs and intracellular EPSCs exhibited PPF. The effects of histamine in this pathway were similar to those in the medial perforant path, but less pronounced (Fig. 4). The fEPSP was decreased to 82·2 ± 6·6% of control ($n = 6$) and the EPSC to 88·1 ± 7·0% of control ($n = 6$). As in the medial perforant path, paired-pulse plasticity was shifted in the direction of facilitation. Histamine (7 μM) enhanced PPF (40 ms ISI) of the fEPSP from 111·7 ± 2·7% in control to 125·9 ± 4·5% ($n = 6$, $P < 0·01$) and PPF of the EPSC from 113·4 ± 3·5% to 126·7 ± 5·0% ($n = 8$, $P = 0·052$).

Pharmacological analysis

To determine on which class of receptors histamine was acting to produce the observed depression of transmission, a number of agonists and antagonists were tested. The specific histamine H_3 receptor agonist *R*-α-methylhistamine dihydrochloride (10 μM) rapidly depressed the medial perforant path fEPSP to 73·4 ± 2·8% of control ($n = 6$) (Fig. 5*A*) and concurrently reduced PPD from 76·0 ± 2·4% in control to 95·2 ± 2·6% ($n = 10$, $P < 0·001$). *R*-α-methylhistamine dihydrochloride was also effective in

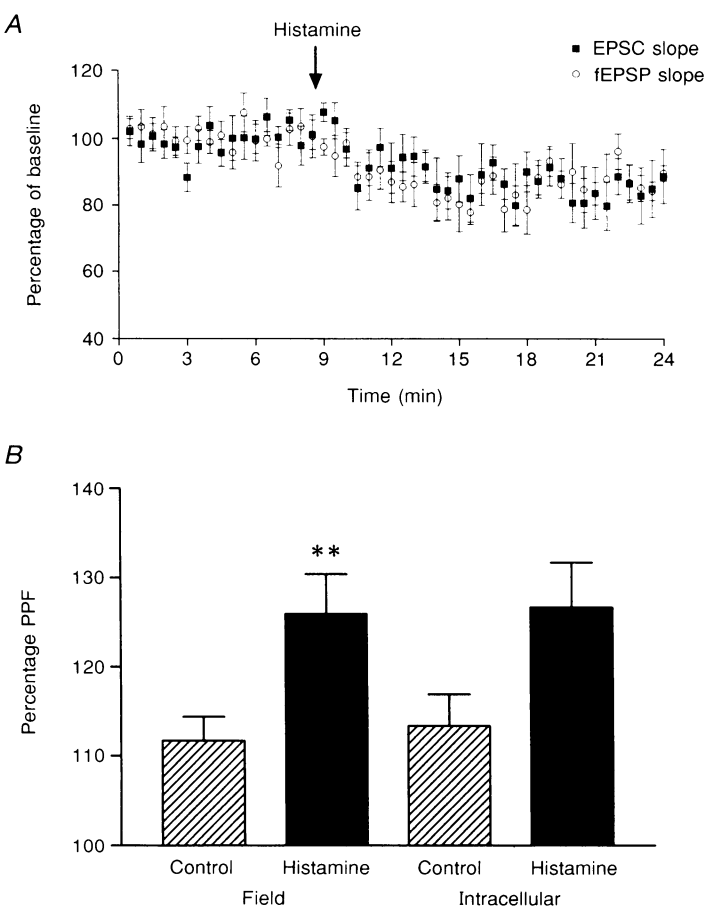

Figure 4. Effects of histamine in the lateral perforant path

A, plot of slope of the EPSC (■), at a holding potential of −75 mV and the fEPSP (○), against time. Histamine (7 μM) was applied at the time indicated by the arrow. *B*, histamine increases paired-pulse facilitation (PPF) in the lateral perforant path. Mean data for the fEPSP ($n = 6$) and the EPSC ($n = 8$). ** $P < 0·01$.

reducing synaptic transmission at 1 μM (22% reduction, $n = 3$) and at 0·1 μM (13% reduction, $n = 3$). In contrast, the H_2 agonist dimaprit (10 μM) did not significantly reduce either the fEPSP (Fig. 5*B*) or PPD ($n = 4$). Application of *R*-α-methylhistamine dihydrochloride (10 μM), in the same slices, reliably depressed synaptic transmission (Fig. 5*B*) and PPD.

Bath application of the H_1 receptor antagonist mepyramine (1 μM, $n = 4$, Fig. 5*E*) or the H_2 receptor antagonist cimetidine (50 μM, $n = 4$, Fig. 5*D*) for 9 min before and 3 min after histamine (7 μM) application did not prevent depression of the fEPSP or the reduction of PPD. However, bath application of the H_3 receptor antagonist thioperamide (10 μM, $n = 6$, Fig. 5*C*), in a similar manner to the other antagonists, completely blocked the depression of synaptic transmission and reduction of PPD by histamine (7 μM). Thioperamide itself had no significant effect on PPD. Addition of histamine, up to 2 h after the switch from thioperamide containing solution to normal solution, did not depress synaptic transmission. This may reflect slow washout of thioperamide from the tissue.

Interaction with presynaptic mGluRs

We were interested to see if the presynaptic histamine heteroreceptors on glutamate-releasing terminals might interact with glutamatergic autoreceptors. Bath application of the class III mGluR agonist AP4 (100 μM) depressed synaptic transmission and reduced PPD, as previously described (Koerner & Cotman, 1981; Kahle & Cotman, 1993; Brown & Reymann, 1995). The fEPSP was depressed to 66·9 ± 3·4% of control following a 15 min application of AP4, at which time point a new steady state had been reached (Fig. 6*A*). This depression is close to the maximum observable (Koerner & Cotman, 1981). Histamine (7 μM) was applied while AP4 was still present. Histamine depressed the fEPSP further to 51·4 ± 2·0% of control ($n = 4$). Taking the values of fEPSP slope immediately before histamine application as 100%, histamine (7 μM) depressed the fEPSP to 76·8 ± 1·9% of control, as

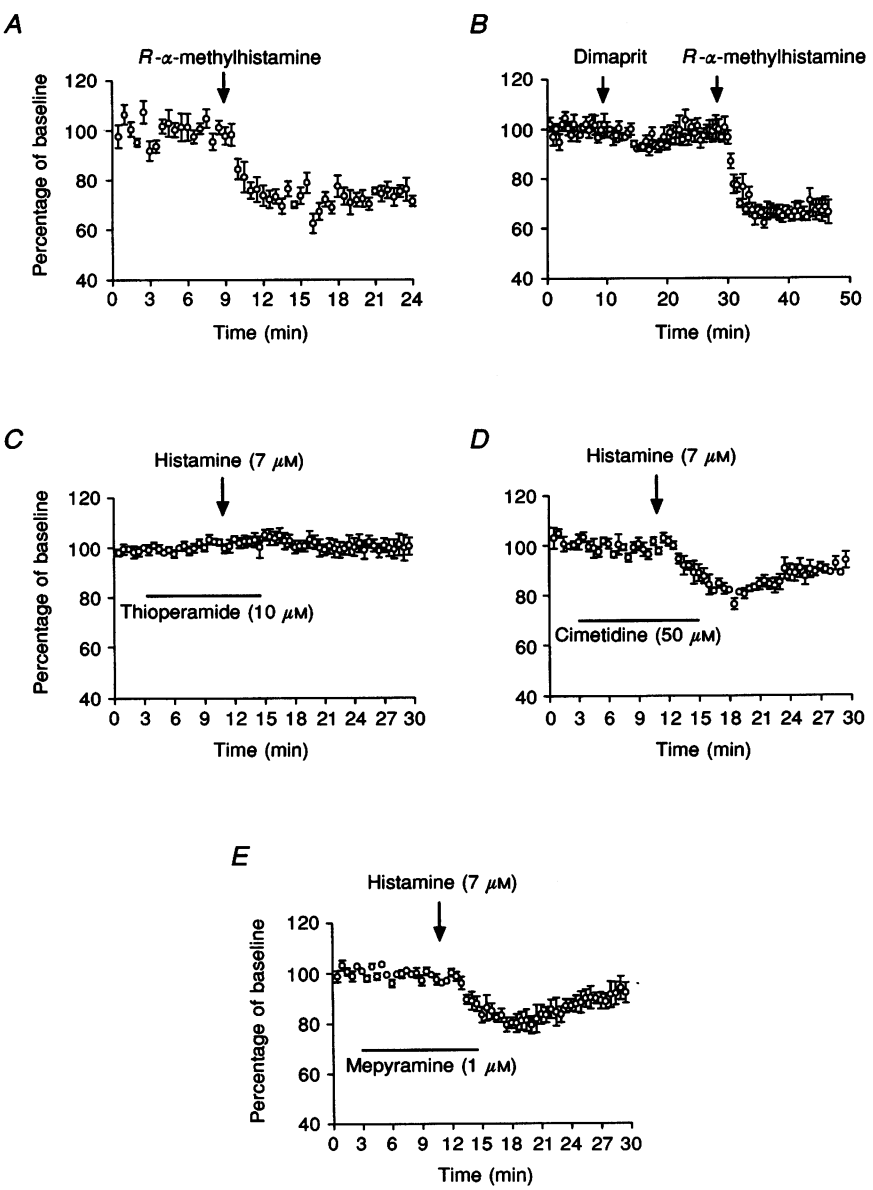

Figure 5. Effects of histamine agonists and antagonists on synaptic responses in the medial perforant path

Plots show the slope of the fEPSP slope against time. Histamine (7 μM), the H_2 receptor agonist dimaprit (10 μM) and the H_3 agonist *R*-α-methylhistamine (10 μM) were administered at the times indicated by the arrows. Antagonists were bath applied for the time indicated by the horizontal bar. *A*, $n = 6$. *B*, $n = 5$. *C*, $n = 6$. *D*, $n = 4$. *E*, $n = 4$.

compared with 75·7 ± 3·2% in the absence of AP4 (see above), i.e. the effects of the two drugs were independent. The effects of the two drugs on PPD were slightly more than additive (Fig. 6*B*). AP4 (100 μM) reduced PPD from 71·4 ± 2·9% in control to 94·1 ± 3·9% ($n = 4$). Histamine (7 μM) further reduced PPD to 112·6 ± 3·7% (i.e. a facilitation of 12·6%). Thus, histamine (7 μM) caused a change in PPD of 18·5% as compared with 13·3% in the absence of AP4.

DISCUSSION

The results presented in this paper suggest that histamine reduces synaptic transmission in the dentate gyrus of the rat by interacting with H_3 receptors located on the presynaptic terminals of perforant path axons. To our knowledge this is the first demonstration that histamine H_3 receptors can regulate glutamate release and is the first electrophysiological study showing an action of histamine on H_3 receptors in the hippocampal formation.

There are several reasons to believe that histamine was acting presynaptically in the present experiments. (i) The synaptic depression caused by histamine was independent of the length of whole-cell recording preceding drug application (up to 30 min) and was independent of the constituents of the pipette solution. Whole-cell recording allows a very effective dialysis of the postsynaptic neuron, and thus the action of postsynaptic histamine receptors is likely to have been disrupted (e.g. see Jahn, Haas & Hatt, 1995). Furthermore, in some experiments the pipette solution contained Cs^+ and QX-314, which block a number of potassium channels (Andrade, 1991) – most postsynaptic actions of histamine involve the opening or closing of potassium channels (Haas, 1992). No consistent changes in holding current or input resistance were observed. (ii) Histamine did not depress postsynaptic responses elicited by pressure ejection of AMPA into the slice. (iii) Histamine reduced PPD in the medial perforant path and increased PPF in the lateral perforant path. An interaction of a drug with PPD or PPF is normally taken to indicate a presynaptic mechanism of action since both PPF and PPD are likely to involve presynaptic mechanisms (Mennerick & Zorumski, 1995). (iv) Histamine did not change the reversal potential of the EPSC. (v) Histamine depressed equally the AMPA and NMDA components of the EPSC, measured as the initial slope and amplitude at 40 ms, respectively. Drugs such as AP4 and *trans*-1-amino-cyclopentane-1,3-dicarboxylic acid (*trans*-ACPD) which are thought to act presynaptically also depress the two components of the EPSC equally (Baskys & Malenka, 1991). Although histamine can act directly at the NMDA receptor, this effect is only observed at high concentrations of histamine (Brown *et al.* 1995) and is strongly dependent on the pH (Yanovsky *et al.* 1995), being negligible at normal pH. These factors may explain why we did not observe a direct effect on the NMDA receptor in this study. (vi) The coefficient of variation for the EPSC was increased in the presence of histamine. According to classical assumptions on the nature of transmitter release this would indicate a decreased probability of transmitter release if the number of sites releasing transmitter remains constant. Taken together, all of these pieces of evidence strongly suggest that histamine depresses synaptic transmission by a presynaptic mechanism. Histamine did not affect the amplitude of the presynaptic fibre volley (see also Greene & Haas, 1990), which reflects the excitability of presynaptic axons. Therefore, it is likely that histamine acts on presynaptic axon terminals and not on the axons themselves.

In this study we have shown that the depression of synaptic transmission by histamine is mediated through H_3 receptors. The highly potent and specific H_3 agonist *R*-α-

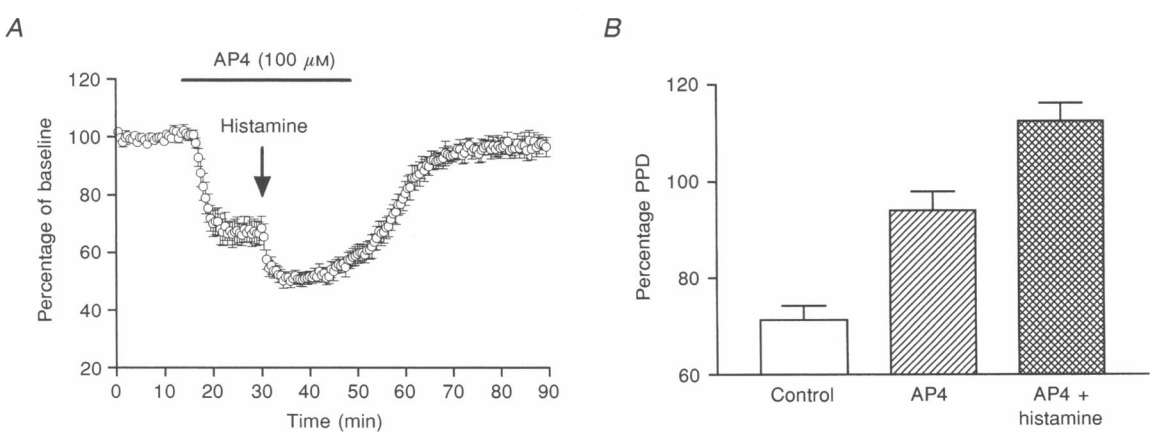

Figure 6. The metabotropic glutamate receptor agonist AP4 and histamine act through independent mechanisms

A, plot of the slope of the fEPSP against time. Histamine (7 μM) was administered at the time indicated by the arrow. AP4 was bath applied for the time indicated by the horizontal bar. *B*, mean data ($n = 4$) showing the reduction of paired-pulse depression (PPD) by AP4 and the further reduction observed in the additional presence of histamine.

methylhistamine mimicked the effects of histamine on synaptic transmission in the concentration range 0·1–10 μM, although it is probable that this drug is active at even lower concentrations since at 100 nM the depression of synaptic transmission was still quite large (13% reduction). This high potency of *R*-α-methylhistamine dihydrochloride clearly points to an action at H_3 receptors (Arrang *et al.* 1987). Furthermore, at 10 μM, the H_3 antagonist, thioperamide blocked the effect of histamine. At this concentration, thioperamide has negligible antagonistic effects on the histamine H_1 and H_2 receptors (Arrang *et al.* 1987). The H_2 agonist dimaprit, the H_2 antagonist cimetidine and the H_1 antagonist mepyramine were all ineffective. These results suggest that the depression of synaptic transmission and reduction of PPD by histamine are due to an action at H_3 receptors. A final confirmation of this conclusion, however, will require full dose–response curves in different concentrations of antagonist, which was not possible with the method of drug application used in this study.

Histamine depressed synaptic transmission more strongly in the medial perforant path than in the lateral perforant path. Although not as striking as the effects of some other neurotransmitters this finding nonetheless emphasizes once more the differential regulation of these two pathways which both synapse on granule cell dendrites. Other more dramatic examples are the stronger depression by the muscarinic agonist carbachol of the medial pathway than the lateral pathway (Kahle & Cotman, 1989) and the effects of noradrenaline, which causes a long-lasting potentiation in the medial perforant path and a long-lasting depression of the lateral perforant path (Dahl & Sarvey, 1989). This differential regulation suggests that the two pathways play quite distinct roles in hippocampal information processing.

Histamine strongly depressed PPD in the medial perforant path. PPD at this synapse consists of two phases (Kahle & Cotman, 1993; Brown & Reymann, 1995): an early phase (up to 100 ms) which can be modulated by mGluR agonists and an mGluR-independent phase (up to 2 s). Whether PPD is due to an intrinsic property of the release process (Mennerick & Zorumski, 1995) or due to feedback of glutamate onto presynaptic autoreceptors (Brown & Reymann, 1995) is at the present time unclear. Due to the lack of knowledge presently available about the mechanism of PPD and the signal transduction mechanism of histamine H_3 receptors it is difficult to suggest on which component of the release process histamine is acting to reduce PPD. However, we can say that the mechanism is likely to be separate from that used by the class III mGluRs, since the class III agonist AP4 did not occlude the effect of histamine. AP4 can inhibit adenylate cyclase and voltage-dependent calcium channels (Pin & Duvoisin, 1995), but it is still unknown if these effects play a role in the inhibition of synaptic transmission and modulation of paired-pulse plasticity. The effect of histamine was not due to an effect on the inhibitory system since $GABA_A$ receptors were blocked by picrotoxin. An effect due to $GABA_B$ receptors is unlikely with this interstimulus interval since $GABA_B$ receptor activation is maximal at later time points (100–200 ms). In addition Kahle & Cotman (1993) found that the $GABA_B$ receptor antagonist phaclofen did not alter paired-pulse depression with a 40 ms interpulse interval. It should also be mentioned that the depression of PPD was not due to the smaller size of the first pulse itself since PPD at this synapse is independent of stimulation strength (Kahle & Cotman 1993; Brown & Reymann, 1995).

What are the functional consequences of H_3 receptor activation in the dentate gyrus? Depression of synaptic potentials in the dentate gyrus will favour information transfer by the direct perforant path input to CA1 rather than through the trisynaptic chain. Indeed it has been suggested by Jones (1993) that this direct pathway is actually more important under normal conditions. This proposal assumes, of course, that histamine does not affect the direct pathway to CA1 in the same way – this will have to be investigated in future studies. In the medial perforant path, the H_3 receptor depresses synaptic transmission after low frequency stimulation but at the same time reduces PPD. Thus, while low-frequency transmission is suppressed, high-frequency transmission will be enhanced. Both synaptic potentials in the dentate gyrus (Winson & Abzug, 1978) and paired-pulse phenomena (Austin, Bronzino & Morgane, 1989) are dependent on behavioural state. Histaminergic neurons fire according to behavioural state and have been strongly implicated in central arousal mechanisms (Lin *et al.* 1988; Wada *et al.* 1991). Thus, together with its other effects on granule cell excitability and the GABAergic system (Greene & Haas, 1990), histamine is likely to be at least partly responsible for behavioural state-dependent modulation of synaptic transmission and plasticity in the dentate gyrus.

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